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On a New Species of *Polycitor* from Japan with Some Remarks on its Mode of Budding [1]

(Ascidiae Compositae)

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With the rise of experimental embryology the relation which holds between cells and an individual has been studied by many workers. The relation between individuals (persons) and a colony (cormus), however, has drawn only little attension from such workers.[2] To take up this latter relation as a problem and solve it, if possible, by experimental methods is the chief aim of the present study.

Our first task was to find out an animal suitable for the study. After some tentative experiments we found in a species of *Polycitor,* which is not uncommon in the vicinity of Simoda Marine Biological Station, a most favourable object. On closer examination the species revealed to be new to science. In the present paper we shall confine ourselves to give a brief description of the species together with some notes on its mode of budding.

REARING OF THE ANIMAL

Before passing to the description, it may not be amiss to spend some words on the rearing of the animal.

To facilitate the handing of the animals and allow an examination of both upper and lower surfaces, the colonies were reared attached to

1) The present investigation was partly aided by the Scientific Research Expenditure of the Department of Education. The author also wishes to express his appreciation of the kind assistance of Mr. Masuo Usui.

2) As an example of recent investigations along this line we may cite Bronstein's paper on the axial gradients in the colony of Bryozoa (C. r. Acad. Sci., Paris, 209, 602–603).

glass slides. The method is not new. At the end of last century Pizon (1899) demonstrated the possibility of rearing the colonies of compound ascidians on a glass slide, but his description was rather simple.[1] In 1903, Bancroft fastened the colonies of *Botryllus* and *Botrylloides* to the slides by tying down the edges, especially those containing the ampullae. According to him one or two days sufficed for forming a firm attachment, when the strings could be removed.

In our species the test is so soft, that tying the colony with strings is practically infeasible. We used gauze instead of strings. Colonies were stripped off from the rock by a spatula and placed on a glass slide. Then they were covered with a small piece of gauze, and the gauze was fastened to the slide with strings. After 3 days the gauze was removed. Some colonies came off, but the remaining ones were firmly attached to the slide. Once attached, the colonies could not be thrown off even by the heaviest storm. Since it was difficult to keep alive the colonies in an aquarium, I set the slides in a wooden frame especially constructed for this purpose, which was then placed in a large fish-preserve.

In August, 1941, we stayed at the station and observed the colonies day by day. From September to November we visited the station once a month. The observation could not be continued further, for the animals were found to have disappeared completely, when examined at the beginning of December.

DESCRIPTION

Polycitor mutabilis,[2] nov. sp.

Colonies are found attached to the under surface of large projecting rocks, at the level of the low-tide mark. A colony is irregularly ellipsoidal in shape, attached to the rock with its flattened base. Its size is variable with localities. At Susaki, Izu, it measures about 1.8 cm. in length, 1.0 cm. in width and 0.6 cm. in height. At Nabeta, where our station is situated, the size is on an average much smaller.

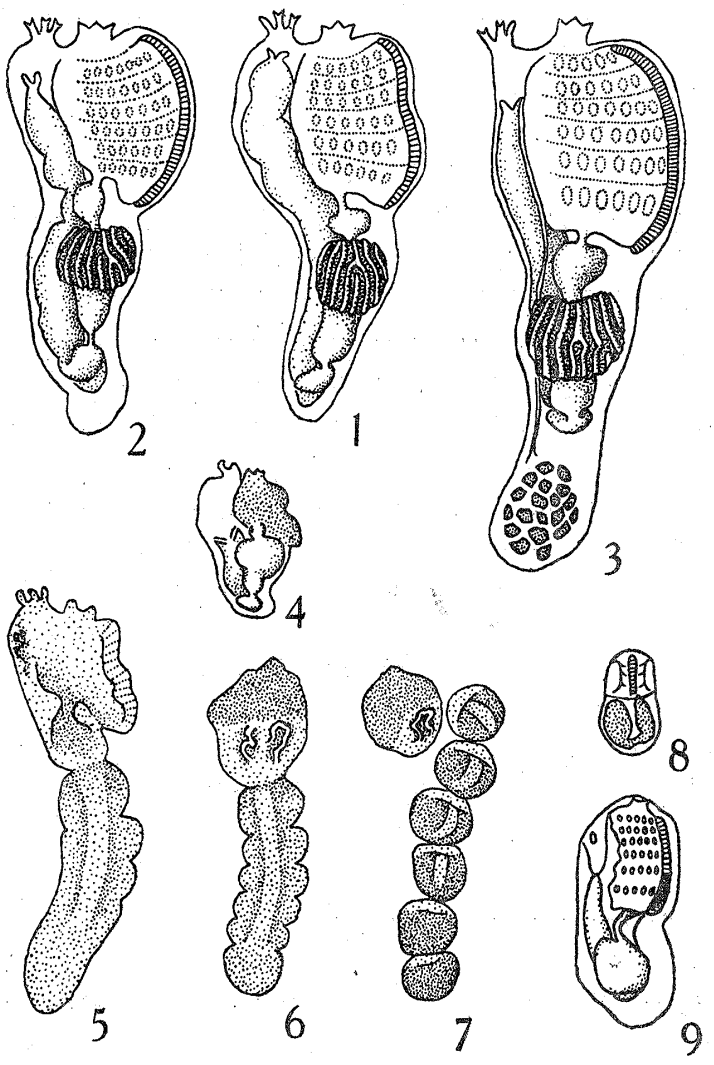
In the natural environment the colony is transparent with a bluish tint. When brought to the laboratory it soon becomes opaque and its colour changes from blue to yellow. This change is reversible, since the colony becomes bluish again when brought back to the sea. The surface of the colony is smooth and free from incrustations.

1) He states simply: ".... j'avais réussi à faire fixer sur une lame de verre." (1899, p. 2).

2) As will be seen, the animal is changeable in more than one respect. Hence the specific name.

There is no arrangement in systems. Ascidiozooids occur at fairly equal distance all through the test and open directly to the exterior each through two apertures.

Ascidiozooids, except those at the margin of the colony, are located perpendicularly, with their ventral side toward the periphery of the colony. They are pale yellowish-brown in colour, measuring about 4 mm. in length.



Polycitor mutabilis, n. sp.

1. Blastozooid in August. ×15. 2. The same in April. ×15. 3. The same in July. ×15. 4. The same in October. ×15. 5–6–7. Strobilization of blastozooid. ×15. 8. Young blastozooid, ventral view. ×15. 9. The same, lateral view. ×30.

Zooids out of the breeding season.—Animals are divided clearly into two parts of almost equal length, viz. thorax and abdomen (fig. 1).

Branchial aperture is clearly six-lobed. Atrial aperture is six-lobed, too, but each lobe is bifurcated at the tip. The degree of bifurcation is variable from individual to individual, and, in a single individual, from lobe to lobe. Sometimes the bifurcation is indicated only by a small notch at the tip of the lobe. Sometimes it extends back to the root of the lobe, so that a lobe is represented practically by two lobes.

There are twelve, six large and six small, tentacles, alternating with another.

The branchial sac is well developed. There are six rows of stigmata. The transverse vessels are accompanied with broad horizontal membranes. The dorsal lamina is represented by triangular languets, six in number.

Owing to the short oesophagus the stomach occupies the anterior portion of the abdomen. Stomach is from spherical to ellipsoidal in shape, its wall being provied with irregular longitudinal folds.

The rectum runs along the left side of the stomach and oesophagus and ends with the two-lobed anus at the base of the atrial syphon.

Zooids of the breeding season.—Animals are divided into three parts, viz. thorax, abdomen and postabdomen (Fig. 3). Thorax and abdomen are not essentially different from those of the animals out of the breeding season. Postabdomen contains the reproductive organs. These are hermaphroditical and consist of many pear-shaped spermatic vesicles and a few large, finely granulated eggs. We have had no chance till now to follow the embryonic development, but it is probable that the embryo completes its development in the atrial cavity.

Localities: Simoda (Sizuoka Prefecture), Misaki (Kanagawa Prefecture), Tateyama (Tiba Prefecture).

SEASONAL VARIATION OF THE ASCIDIOZOOID

The complete life-history of the animal is still unknown.

What we have been able to ascertain till now is the fact, that the ascidiozooid undergoes considerable morphological changes in the course of one year.

In the spring the animal has a short postabdomen, in which the reproductive organs are not yet developed (fig. 2). Animals increase in number by strobilization (see below).

In July the animal is provided with a well-developed postabdomen, filled with spermatic vesicles and mature eggs (fig. 3). Sexual reproduction seems to be restricted to this month.

In August the animal has no postabdomen (fig. 1). August to September is the season, when the budding of ascidiozooids and the polymerization of colonies occur most vividly. The test is gelatinous. A single colony becomes more than twenty within a month.

In the autumn the animal begins to undergo involution. Polymerization of colonies does not occur any more. The colony as a whole becomes comparatively higher. The test becomes tenacious in consistency, and a septum appears between each two neighbouring zooids, so that the colony now becomes an aggregate of compartments. At the same time zooids undergo involution and become much smaller (fig. 4). Whether this is a sign of the coming death or the beginning of hivernation could not be decided, for, from some unknown cause, the colonies on the glass slides disappeared suddenly leaving no traces behind.

Systematic position of our species

So far as I know, only two species of *Polycitor* from Japan are known by their scientific names,[1] though A. Oka mentions in his synopsis of Japanese ascidians (1935) at least 7 species as occurring in Japan.

Among all known species of *Polycitor*, *Polycitor proliferus* (Oka) most closely resembles our species. The original description of the former is almost wholly applicable to the latter, except that in our species the stomach is not "completely smooth", but folded.

The body of a *Polycitor* is generally divided into two parts, viz. thorax and abdomen. That is true of our species, if we take an animal out of the breeding season. In the breeding season, however, the postabdomen becomes very conspicuous, so that the whole body consists of three parts, viz. thorax, abdomen and postabdomen. But this postabdomen does not contain the heart, but contains only the reproductive organs, thus differing from the postabdomen of Polyclinidae. In a sense our species takes an intermediate position between Polycitoridae and Polyclinidae, like such genera as *Sigillina*, *Sigillinaria*, etc.

Budding of the colony

Polycitor proliferus is known for its peculiar mode of budding. One or two individuals, set free from the mother colony, establish a new

1) *Polycitor parvus* (Oka, 1927) and P. *proliferus* (Oka, 1933).

colony, when settled on a new ground. Thus A. Oka speaks of a colonial budding. He argues further that the relatively small size of each colony may possibly depend on this peculiar mode of budding.[1] In our species, too, each colony is relatively small. But I have never seen, in nature at least, that a colony propagates by colonial budding. I am rather inclined to believe, that the colonial budding, such as described by A. Oka, is an unnatural phenomenon, caused by an undue treatment either before or after preservation.[2] In our species the small size is rather due to the fact, that a colony from a single embryozooid, instead of remaining as such, divides itself into smaller colonies by means of polymerization. Starting from a single colony reared on a glass slide we get in the course of one month more than a dozen colonies. What is comparable to a large colony of other compound ascidians is, therefore, not a single colony, but a mass of colonies. I should like to name such colonies as those of our species "cormomeres". A cormomere is a unit, which is higher than an individual (person), but lower than a colony (cormus).

The process of polymerization will be discussed in detail in a later paper.

BUDDING OF THE BLASTOZOOID

Budding of the embryozooid is still to be investigated.

Budding of the blastozooid takes the same course as in *Aplidium* (cf. Brien, 1925). As budding approaches, the blastozooid undergoes a kind of degeneration, and, at the same time, a constriction appears at the level of the junction between oesophagus and stomach, thus dividing the animal roughly into thorax and abdomen (fig. 5). The thoracic part contains the branchial sac, oesophagus, a part of epicardium, and the terminal part of the rectum, while the abdominal part contains the stomach, mid-intestin, heart, a part of epicardium, and the proximal part of the rectum. Then the abdominal part is divided into six successive regions by deep constrictions (fig. 6). Finally the abdominal part becomes completely separated from the thoracic, and, at the same time, splits into six young ascidiozooids (fig. 7). These come later to the surface of the colony and complete their development (fig. 8 and 9). Since the abdominal part, which gives rise to new ascidiozooids, contains the digestive tract and the epicardium, the budding should be termed "entero-

1) "Dass die Kolonien alle verhältnismässig sehr klein bleiben, dürfte wohl auf diese eigentümliche Vermehrungsweise der Kolonien zurückzuführen sein." (Oka, 1933, p. 438)

2) The material, which served to Dr. A. Oka as the basis of his argumentation, was collected and preserved at Keelung, Formosa, by a field naturalist.

epicardial". The degenerated thorax is supposed to regenerate its lost part.

In *Aplidium* the postabdomen is reduced to a minimum before the strobilization takes place. Such is not the case in our species, for the postabdomen is from the outset very small, if not lacking at all.

The original polarity is conserved also in young ascidiozooids. Their antero-posterior axis and left-right axis coincide with those of the parent zooid. Thus the following view expounded by N. J. Berill gets a new support: Whereever organic continuity is maintained between bud and parent, the polarity of the bud is a derivative of that of the parent (cf. Berill, 1941).

There exists an axial gradient along the long axis of the body. The constriction at first appears at the anterior end of the abdomen and proceeds posteriorly. When strobilization occurs, each anterior bud is more advanced in development than its posterior one.

The genus *Aplidium* belongs to Polyclinidae. The fact that the budding in our species takes the same course as in *Aplidium* adds a new instance to support the view, that our species takes an intermediate position between Polycitoridae and Polyclinidae.

Summary

The present paper deals with *Polycitor mutabilis*, a new species of compound ascidians from Japan.

This species is interesting from following points:

1. Zooids undergo morphological changes in the course of one year. In the breeding season, the body is divided into three parts viz. thorax, abdomen and postabdomen. The postabdomen does not contain the heart, but contains only the reproductive organs. In other seasons the body is divided into two parts viz. thorax and abdomen.

2. Colonies propagate by a kind of polymerization. A single colony becomes more than a dozen colonies within a month.

3. Blastozooids increase in number by an "entero-epicardial" budding, analogous to that of *Aplidium*.

4. That the zooids have, though temporarily, a clear postabdomen and propagate, like *Aplidium*, by an entero-epicardial budding leads us to suppose that this species links two faminlies: Polycitoridae and Polyclinidae.

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